CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

THE EFFECTS OF CARMEDA BIOACTIVE SURFACE ON HUMAN BLOOD COMPONENTS DURING SIMULATED EXTRACORPOREAL CIRCULATION

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Postoperative morbidity after cardiopulmonary bypass most commonly manifests as bleeding diatheses or pulmonary dysfunction. The pathophysiology has been attributed to the activation of cellular and humoral components of blood after contact with an artificial surface. Development of a surface that would be nonthrombogenic and also would constitute a less potent inflammatory stimulus would therefore be beneficial. In the following experiments, we evaluated the heparin-bonded Carmeda Bioactive Surface (Medtronics Cardiopulmonary, Anaheim, Calif.) in an in vitro model of extracorporeal circulation at standard-dose heparin (5 U/ml), to examine the effects of the surface treatment on activation of blood elements, and at reduced-dose heparin (1 U/ml), to determine whether surface-bound heparin would serve as an effective anticoagulant. During the initial recirculation period, platelet counts in the Carmeda ($n = 12$) circuits were preserved at both doses of heparin and compared with control values ($n = 12$): At 5 U/ml, control 36% ± 4% (mean ± standard error of the mean) versus Carmeda 81% ± 5%; at 1 U/ml, 43% ± 3% versus 61% ± 10%, expressed as a percent of baseline at 30 minutes, $p < 0.05$. Furthermore, plasma levels of platelet factor 4 and β-thromboglobulin were significantly reduced in the Carmeda circuits throughout the experiment: At heparin 5 U/ml, 2500 ± 340 ng/ml versus 604 ± 191 ng/ml; at 1 U/ml, 2933 ± 275 ng/ml versus 577 ± 164 ng/ml of platelet factor 4 at 2 hours ($p < 0.05$). The pattern of β-thromboglobulin release was similar, with effects more pronounced at the lower dose of heparin. Surface modification also reduced leukocyte depletion ($p < 0.05$) and release of elastase at both concentrations of heparin (5 U/ml, 0.72 ± 0.29 ng/ml versus 0.33 ± 0.23 ng/ml; 1 U/ml, 0.85 ± 0.08 ng/ml versus 0.20 ± 0.05 ng/ml, at 2 hours, $p < 0.05$). Moreover, as heparin concentration was reduced, Carmeda surface treatment significantly decreased generation of C3a des Arg (1 U/ml, 14,410 ± 3558 ng/ml versus 3053 ± 1039 ng/ml at 2 hours, $p < 0.05$). Although heparin bonding was originally intended to obviate the need for systemic heparinization, Carmeda treatment did not reduce fibrinopeptide A generation at the lower dose of heparin. In summary, Carmeda treatment failed to exhibit anticoagulant efficacy in this model; however, the data suggest that surface modification may have a role in ameliorating the typical inflammatory response initiated by blood contact with an artificial surface. (J Thorac Cardiovasc Surg 1996;111:1073-84)

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The evolution of cardiac surgery over the past four decades has been accompanied by a greater dependence on extracorporeal circulation.[1] Although the bypass circuit has undergone extensive modifications since its inception, the sequelae of blood-surface interactions and high-dose heparin therapy continue to plague cardiac surgeons. The clinical complications are primarily related to bleeding diatheses, to the initiation of a systemic inflammatory response, or to both. Hemostatic function is impaired by stimulation of the intrinsic coagulation system, thrombocytopenia, and platelet dysfunction.[2, 3] Continued reliance on high-dose heparinization not only may promote perioperative bleeding, but also incurs the risks associated with protamine reversal.[4] Neutrophil activation, in part mediated by the complement system, contributes to postoperative pulmonary dysfunction and prolonged ventilator dependence in susceptible patients.[5-7] More recently, interactions of the formed blood elements, both with each other and with the complement and coagulation systems, have begun to define the complexity of the surface-induced response.

Biomaterials research has been directed toward the development of a nonthrombogenic surface for use during mechanical circulatory support. A number of efforts have focused on heparin surface bonding[8] with the intention of eliminating or reducing the need for systemic heparinization. Presentation of blood to the surface has been shown to initiate inflammatory, hemostatic, fibrinolytic, and cellular cascades.[9] Moreover, this type of surface modification may alter these responses and thus prevent some of the observed clinical complications.

We evaluated the effects of a covalently bound heparin surface treatment, Carmeda Bioactive Surface (Medtronic Cardiopulmonary, Anaheim, Calif.), in a well-established in vitro model of cardiopulmonary bypass. These studies were performed at full heparinization (a 5 U/ml dose of heparin) to determine if heparin surface treatment altered the pattern of platelet, leukocyte, and complement activation during simulated cardiopulmonary bypass. In addition, fibrinopeptide A levels were used to directly assess the anticoagulant efficacy of this surface modification during contact with human blood, after reduction of the exogenous heparin dose (1 U/ml dose of heparin).

Materials and methods

Perfusion circuits. Perfusion circuits with a surface area of 0.9 m^2 were assembled from standard medical-grade polyvinylchloride tubing, polycarbonate connectors, venous reservoir bags, and a 0.8 m^2 pediatric reversed hollow-fiber membrane oxygenator (Minimax, Medtronic). The entire circuit, including the venous reservoir bag, tubing, and oxygenator, was flushed with 100% carbon dioxide for 15 minutes before priming. Then 400 ml of blood from a single donor was drawn directly into the venous reservoir bag containing porcine lung heparin in a dose of either 5 or 1 U/ml and 1.65 gm of glucose. The group containing 5 U/ml approximated the standard heparin dose used in clinical cardiopulmonary bypass. The reduced heparin dose, 1 U/ml, was chosen because it represented the lowest concentration of heparin that allowed recirculation through the simulated loop without formation of macroscopic clots. After removal of residual carbon dioxide gas from the venous reservoir bag, the remaining components of the circuit were primed by permitting the blood to displace the carbon dioxide in the tubing and oxygenator under gravity flow. Blood was recirculated by a precisely shimmed, barely occlusive calibrated double-roller pump (Sarns, Inc., Ann Arbor, Mich.) for 2 hours at a rate of two times the circulating volume in liters per minute.[10] A minimum volume of 200 ml was required for recirculation in this circuit, and consequently no additional volume was necessary to maintain the specified conditions of the model. Blood temperature was maintained at 37° C. The oxygenator was ventilated with a 95% oxygen/5% carbon dioxide mixture at a rate of 1 L/min. For the experimental group, the surfaces of the oxygenator and all components of the perfusion circuit were composed of heparin-bonded materials (Carmeda). With the exception of the surface treatment, the control circuits were identical.

Blood acquisition and sampling protocol. These studies were approved by the Temple University Committee on Human Investigations and the National Institutes of Health. Written and verbal consent was obtained from each volunteer before venipuncture.

Blood for recirculation trials was drawn from volunteers who abstained from aspirin and aspirin-containing medications for at least 2 weeks before venipuncture. Donors met Red Cross standards for blood donation, including a hematocrit value greater than 37%. All blood samples were drawn into syringes containing 3.8% trisodium citrate (9:1 vol/vol). Three 20 ml baseline samples were obtained directly from the volunteer and were incubated at 37° C. Additional 25 ml samples were withdrawn from the circuit at 5 minutes, 1 hour, and 2 hours of recirculation and analyzed with the aforementioned baseline samples. All samples were assessed immediately for platelet and leukocyte number and platelet reactivity to adenosine diphosphate (ADP). Samples to be assayed for platelet and leukocyte activation products, complement C3a des Arg (C3a), and fibrinopeptide A were processed immediately and frozen at −70° C for later analysis. Additional 10 ml blood samples were obtained at 15 and 30 minutes of recirculation for leukocyte and platelet counts and fibrinopeptide A determinations.

Platelet studies

Platelet counts. Whole blood platelet counts were obtained with a Coulter ZBI Counter (Coulter Electronics, Inc., Hialeah, Fla.).

Platelet aggregation. Platelet-rich plasma was prepared from citrated whole blood by centrifugation at 150 *g* for 10

minutes at 25° C. After gentle aspiration of the platelet-rich plasma (350,000 ± 50,000/μl), the remaining blood was centrifuged at 12,000 *g* for 5 minutes at 25° C in a microcentrifuge to obtain platelet-poor plasma with a platelet count less than 1000/μl.

Platelet aggregation studies were performed as described previously.[11] We determined that the platelet release reaction was complete when ADP-induced aggregation exceeded 62% light transmission through platelet-rich plasma at 5 minutes. The threshold concentration of aggregating agent was defined as the lowest logarithmic concentration necessary to produce irreversible or second-wave aggregation, indicating that platelet granule release has occurred. This value was determined for the control baseline samples and used to test the responsiveness of the recirculated platelets at each corresponding time point. Reactivity to ADP at the recirculation time point divided by its control sample was then expressed as percent alteration. Normal platelet reactivity was demonstrated in control baseline samples for all studies using ADP in concentrations of 1 to 5 μmol/L.

Platelet factor 4 and β-thromboglobulin. Citrated whole blood in 3 ml aliquots was transferred to plastic tubes containing 10% disodium ethylenediaminetetraacetic acid (Na_2 EDTA), theophylline (5.4 mg/ml), and prostaglandin E_1 (3×10^{-3} mol/L) and immediately centrifuged at 2000 *g* for 20 minutes at 4° C to obtain platelet-poor plasma. The platelet-poor plasma was again centrifuged at 12,000 *g* at room temperature for 2 minutes in a microcentrifuge.

Plasma levels of platelet factor 4 and β-thromboglobulin were quantitated by radioimmunoassay with specific tracers and antibodies (Abbot Laboratories, Chicago, Ill., and Amersham Corporation, Arlington Heights, Ill.), as previously described.[13, 14] The sensitivity of these assays is 1 ng/ml (platelet factor 4) and 10 ng/ml (β-thromboglobulin).

Thromboxane B_2. Aliquots (3.7 ml) of citrated whole blood were transferred to a plastic tube containing Na_2 EDTA for a final concentration of 10 mmol/L and spun for platelet-rich plasma. Before high-speed centrifugation (2000 *g* at room temperature for 10 minutes) to prepare platelet-poor plasma, enough indomethacin dissolved in 100% ethyl alcohol was added to give a final concentration of 10 μmol/L.[15]

Plasma levels of thromboxane B_2 were measured by radioimmunoassay with a specific tracer and antibody (New England Nuclear/Dupont, Boston, Mass.).[16] The sensitivity of this assay is 25 pg/ml.

Leukocyte count. White blood cell counts were performed on a Coulter ZBI Counter.

Neutrophil elastase. Plasma for determination of release of neutrophil elastase was prepared by centrifuging citrated whole blood at 2000 *g* for 20 minutes at 4° C.

Neutrophil elastase levels were immunochemically quantitated (EM Diagnostics, Gibbstown, N.J.) by a heterogeneous sandwich enzyme-linked immunosorbent assay for the elastase–α_1–proteinase inhibitor complex.[17] In brief, neutrophil elastase–α_1–proteinase inhibitor complex from known standards and plasma samples was incubated at room temperature and allowed to bind to antibody-coated tubes by its neutrophil elastase end. A second antibody marked with alkaline phosphatase was then added, complexing with the exposed α_1–proteinase inhibitor end. The enzyme activity of the immunocomplexed alkaline phosphatase was measured spectrophotometrically at A405. The colored product is directly proportional to the concentration of neutrophil elastase–α_1–proteinase inhibitor. All unknown samples were read from a generated standard curve.

C3a. C3a was determined in plasma that was rapidly processed by centrifugation of citrated whole blood at 2000 *g* for 20 minutes at 4° C.

Before quantitation of C3a by radioimmunoassay (Amersham Corporation), plasma was pretreated to prevent interference from the precursor C3. Both standards and unknown samples were allowed to react at room temperature for 30 minutes with highly specific tracer and antiserum. Separation of the antibody-bound fraction from the free fraction was effected with addition of a second antibody. After an additional 30-minute incubation, separation of this antibody-bound fraction was achieved by the addition of isotonic saline solution and high-speed centrifugation in the cold. The sensitivity of this assay is 40 ng/ml.[18, 19]

Fibrinopeptide A. Three milliliters of citrated whole blood was added to an anticoagulant containing aprotonin to prevent spurious generation of fibrinopeptide A during the preparation of platelet-poor plasma by centrifugation at 2000 *g* for 20 minutes at 4° C. Before performance of the radioimmunoassay, plasma was treated with bentonite to remove plasma fibrinogen and prevent cross-reactivity with the antibody.

Fibrinopeptide A (ByK-Sangtec Diagnostica, Dietzenbach, Germany) from standards and plasma samples competed with radioactive-labeled fibrinopeptide A and antiserum. The antibody was precipitated with bound tracer by using a second antibody in combination with polyethylene glycol.[20] The amount of radioactivity bound to the second antibody was then measured. The lower limit of sensitivity for this assay is 1 ng/ml.

Statistics. Mean, standard deviation, and standard error of the mean were calculated for each determinant at each sampling point. Univariate and multivariate analyses of variance were used for comparison between groups over time. One-way analysis of variance with the Tukey honest significant difference (HSD) post-hoc test was used to evaluate specific time points. Kruskal-Wallis one-way analysis of variance was used to examine platelet aggregation and thromboxane B_2 data. Unless otherwise indicated, all statistical results are based on *p* values obtained by univariate and multivariate analyses of variance. A *p* value of less than 0.05 was considered significant.[21] Six experiments were performed in each group.

Results

Platelet counts. Mean platelet counts are expressed as a percentage of the initial platelet count obtained from baseline samples drawn directly from the volunteers (Fig. 1).

For control circuits using heparin at a concentration of 5 U/ml, platelet count declined to 36% ± 5% (mean ± standard error of the mean) of baseline at

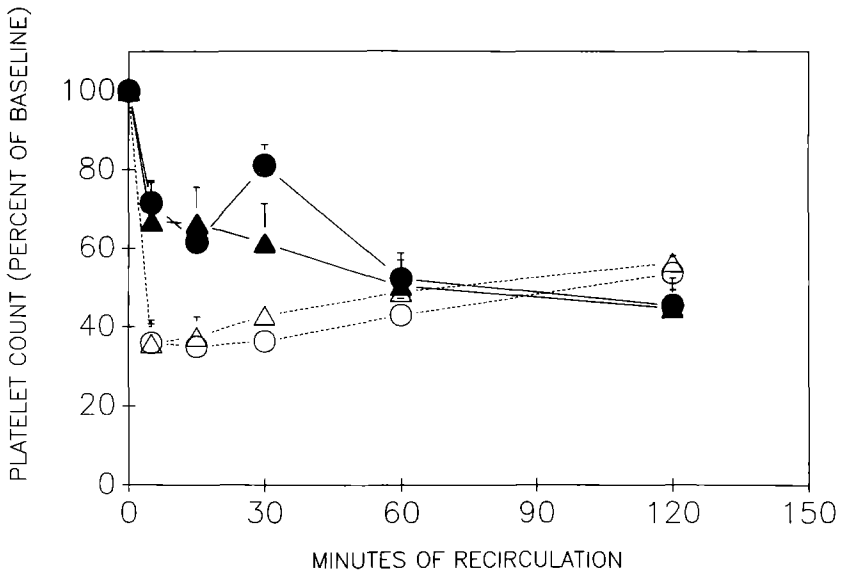

Fig. 1. Circulating platelet count. Mean platelet counts from whole blood are expressed as a percentage of baseline and are plotted against time. *Open circle,* heparin 5 U/ml control ($n = 6$); *closed circle,* heparin 5 U/ml coated ($n = 6$); *open triangle,* heparin 1 U/ml control ($n = 6$); *closed triangle,* heparin 1 U/ml coated ($n = 6$).

Table I. *Alteration of ADP-induced platelet response*

Heparin dose	*Experimental group*	*Time in minutes*		
		5	*60*	*120*
5 U/ml	Control	72% ± 7%	68% ± 8%	68% ± 4%
	Carmeda	40% ± 6%	68% ± 5%	63% ± 9%
1 U/ml	Control	72% ± 9%	79% ± 6%	78% ± 5%
	Carmeda	44% ± 9%	58% ± 12%	60% ± 8%

Values presented are means ± standard error of the mean. These data represent alteration (loss) of responsiveness of recirculated platelets relative to control platelets. See *Materials and methods.* For all time points, $n = 6$.

5 minutes. Platelet counts remained depressed at 2 hours, with a value of 54% ± 4%. In the treated circuits, the platelet counts were maintained at 72% ± 6% of baseline after 5 minutes of extracorporeal circulation. After 2 hours, the platelet counts decreased to levels similar to those of the untreated circuits (46% ± 7%, $p < 0.05$ vs control).

In the group with reduced exogenous heparin (1 U/ml), the control circuit platelet counts decreased to 36% ± 6% of baseline at 5 minutes of recirculation, with a value of 56% ± 2% at 2 hours. Platelet counts at corresponding time points in the heparin-coated circuits were 67% ± 10% and 45% ± 5% ($p < 0.05$ vs control).

Thus at a 5 U/ml heparin dose, surface treatment reduced platelet depletion through the initial 30-minute recirculation period. In contrast, with a 1 U/ml heparin dose, platelet loss was reduced only at 15 minutes ($p < 0.05$ vs control by the Tukey HSD post-hoc test).

Platelet aggregation. The data depicted in Table I represent the platelet response to ADP-induced aggregation.

Heparin coating preserved platelet reactivity early in the protocol, as assessed by ADP-induced aggregation in the 5 U/ml heparin group ($p < 0.05$ vs control at 5 minutes by Kruskal-Wallis one-way analysis of variance). However, at reduced-dose heparin, statistical significance was not obtained although a trend toward early preservation of platelet function was noted.

Platelet factor 4 and β-thromboglobulin. In the 5 U/ml heparin control group, the baseline level of platelet factor 4 was 25 ± 4 ng/ml (Fig. 2). After 5

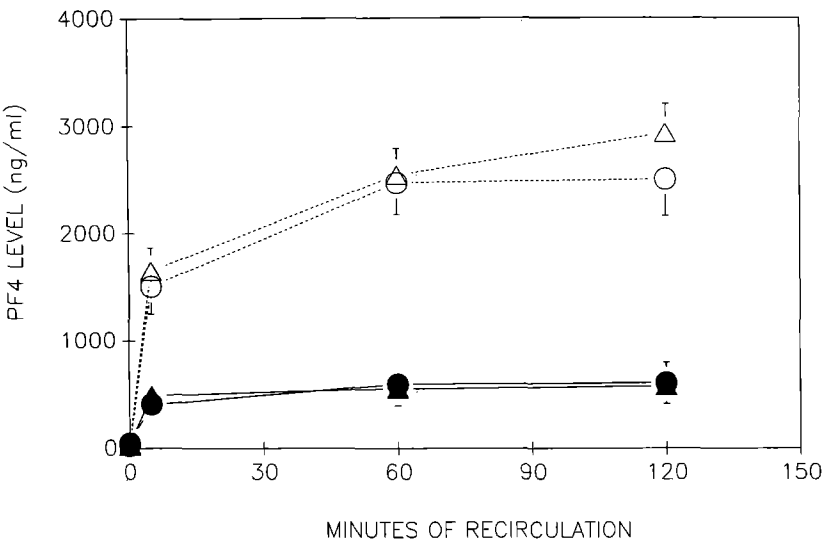

Fig. 2. Platelet factor 4. Mean plasma levels of platelet factor 4 *(PF4)* (mean ± standard error of the mean) are plotted against time and expressed in nanograms per milliliter. Otherwise legend is identical to that of Fig. 1.

minutes of extracorporeal circulation, this value rose to 1505 ± 254 ng/ml and reached a plateau at 2500 ± 340 ng/ml by 2 hours. Surface modification attenuated this rise in plasma platelet factor 4 levels, with a baseline of 46 ± 12 ng/ml increasing to 409 ± 103 ng/ml and 604 ± 191 ng/ml by 5 minutes and 2 hours, respectively ($p < 0.05$ vs control).

In the control group using a 1 U/ml heparin dose, platelet factor 4 increased from a baseline value of 24 ± 3 ng/ml to 1650 ± 219 ng/ml after 5 minutes of recirculation, rising to 2933 ± 275 ng/ml at 2 hours. Platelet factor 4 release was markedly reduced in the experimental circuits, with a baseline level of 37 ± 7 ng/ml and values of 492 ± 86 ng/ml at 5 minutes and 577 ± 164 ng/ml at 2 hours ($p < 0.05$ vs control).

Because platelet factor 4 possesses an affinity for heparin, the possibility of platelet factor 4 binding to the heparin surface treatment was addressed by corroborating the data with measurements of β-thromboglobulin, another α-granule marker. In the uncoated circuits with standard heparin dose (5 U/ml), the initial plasma β-thromboglobulin levels (Fig. 3) rose from 70 ± 11 ng/ml to 2040 ± 260 ng/ml after a 5-minute recirculation period, further increasing to 3367 ± 606 ng/ml at 2 hours. In contrast, β-thromboglobulin in the treatment group only rose from 132 ± 56 ng/ml to 788 ± 178 ng/ml and 1167 ± 253 ng/ml after 5 minutes and 2 hours, respectively ($p < 0.05$ vs control).

At the 1 U/ml heparin dose, β-thromboglobulin in the control group increased from 51 ± 5 ng/ml to 3208 ± 492 ng/ml after 5 minutes of recirculation. β-thromboglobulin levels peaked at 2 hours with a value of 5700 ± 1109 ng/ml. Release of β-thromboglobulin was again attenuated by the heparin surface treatment, with an initial value of 83 ± 13 ng/ml rising to only 1207 ± 254 ng/ml at 5 minutes and 1872 ± 681 ng/ml at 2 hours ($p < 0.05$ vs control).

Thus surface treatment was associated with a marked and sustained reduction of platelet α-granule release at both doses of heparin.

Thromboxane B_2. The data in Table II demonstrate minimal generation of thromboxane in coated circuits at either dose of heparin. In the control circuits, the trend was toward generation of greater levels of thromboxane, particularly at the reduced heparin dose (1 hour and 2 hours, $p < 0.05$ vs control by Kruskal-Wallis one-way analysis of variance). Time points examined in the 5 U/ml heparin groups were not significantly different.

Leukocyte count and neutrophil elastase. Baseline leukocyte counts in untreated circuits with 5 U/ml heparin (Fig. 4, *A*) were 6.7 ± 0.5 × $10^3/mm^3$. Over the ensuing 2 hours of simulated extracorporeal circulation, the leukocyte counts gradually declined to 4.9 ± 0.4 × $10^3/mm^3$. In the heparin-treated circuits, leukocyte counts were preserved with an initial value of 6.1 ± 0.7 × $10^3/mm^3$ and

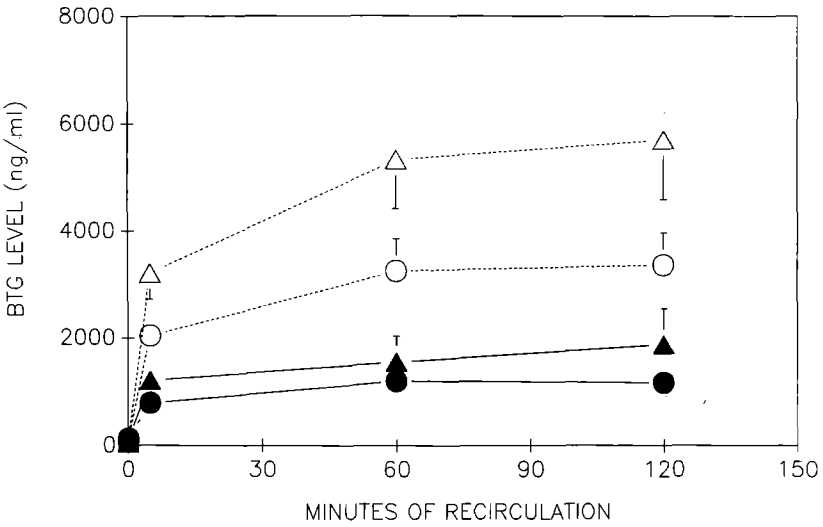

Fig. 3. β-Thromboglobulin. Mean plasma levels of β-thromboglobulin *(BTG)* (mean ± standard error of the mean) are plotted against time and expressed in nanograms per milliliter. Otherwise legend is identical to that of Fig. 1.

Table II. *Thromboxane B_2 generation*

Heparin dose	*Experimental group*	*Time in minutes*			
		0	*5*	*60*	*120*
5 U/ml	Control	426 ± 56	353 ± 693	12,118 ± 7,041	13,469 ± 7,629
	Carmeda	617 ± 175	676 ± 222	980 ± 323	944 ± 307
1 U/ml	Control	333 ± 94	3,997 ± 3,122	31,466 ± 18,817	29,916 ± 16,293
	Carmeda	779 ± 236	588 ± 191	2,615 ± 968	3,035 ± 1,529

Values presented are means ± standard error of the mean, expressed in picograms per milliliter. For all time points, $n = 6$.

counts of 5.5 ± 0.6 × 10^3/mm^3 noted at 2 hours ($p < 0.05$ vs control).

When blood was recirculated with 1 U/ml heparin in the control group (Fig. 4, *B*), a relative leukopenia was observed over time, with counts decreasing from 6.8 ± 0.4 × 10^3/mm^3 to 4.2 ± 0.3 × 10^3/mm^3 at 2 hours. In contrast, heparin coating maintained circulating leukocyte counts (baseline: 5.9 ± 0.4 × 10^3/mm^3 and 2 hours: 5.2 ± 0.4 × 10^3/mm^3; $p < 0.05$ vs control).

When blood containing 5 U/ml heparin was recirculated through uncoated circuits, plasma neutrophil elastase (Fig. 5) rose from an initial value of 0.06 ± 0.02 ng/ml to 0.72 ± 0.29 ng/ml at 2 hours. In comparison, heparin surface treatment reduced neutrophil elastase release, with a baseline of 0.05 ± 0.03 ng/ml increasing to only 0.33 ± 0.23 ng/ml at 2 hours ($p < 0.05$ vs control).

Neutrophil elastase levels in the control group at reduced heparin dose (1 U/ml) rose from 0.06 ± 0.02 ng/ml to 0.85 ± 0.08 ng/ml at 2 hours. When circuits were coated with heparin, there was a significant reduction in neutrophil elastase release (baseline: 0.07 ± 0.01 ng/ml; 2 hours: 0.20 ± 0.05 ng/ml; $p < 0.05$ vs control).

Thus surface treatment was found to reduce both leukocyte depletion and elastase release at both doses of heparin.

C3a. Although other investigators have demonstrated that complement generation may be manifested by the formation of terminal complement complexes,[22] the validity of C3a measurement as an indicator of complement activation appears to be well founded.[23]

C3a (Fig. 6) increased from an initial level of 240 ± 67 ng/ml to 5630 ± 964 ng/ml at 2 hours in control circuits with 5 U/ml heparin doses. In the treatment group, C3a levels also increased from a baseline of 130 ± 13 ng/ml to 3933 ± 797 ng/ml at 2 hours ($p > 0.05$ vs control).

With reduced heparin (1 U/ml), the untreated circuits demonstrated a profound increase in C3a

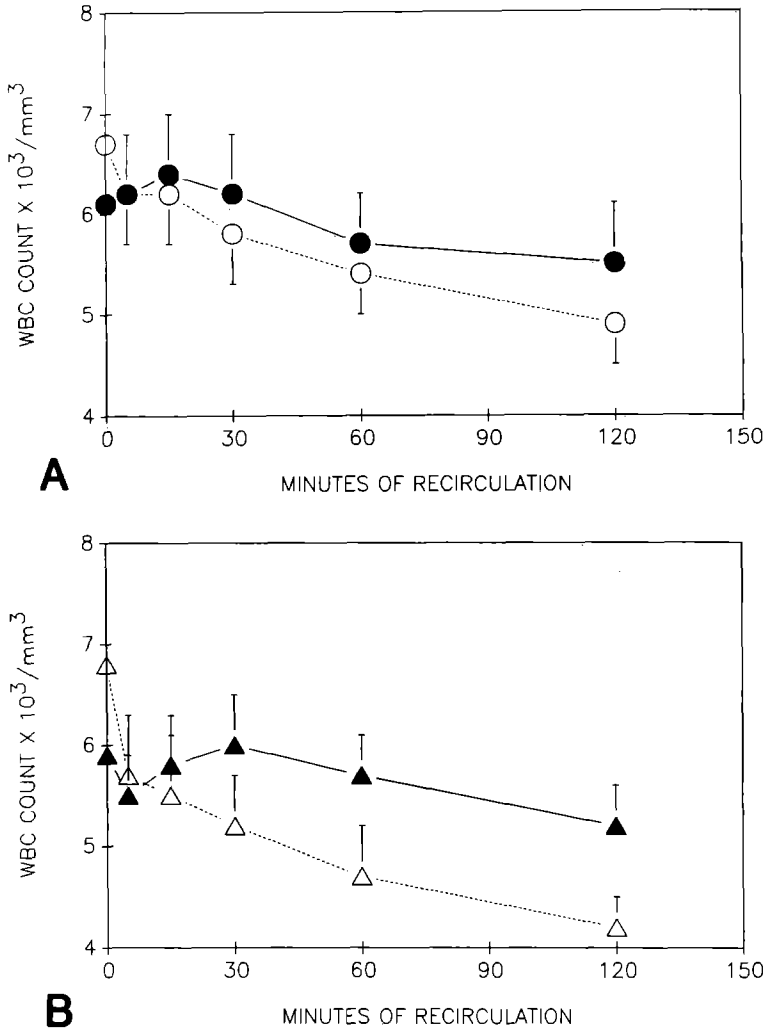

Fig. 4. A and **B,** Circulating leukocyte count. Mean leukocyte *(WBC)* counts (mean ± standard error of the mean) from whole blood are plotted against time and expressed × $10^3/mm^3$. Otherwise legend is identical to that of Fig. 1.

production. The baseline value rose from 175 ± 29 ng/ml to 14,410 ± 3,558 ng/ml after 2 hours of recirculation. This response was significantly attenuated in the heparin-coated circuits. Before recirculation, C3a measured 166 ± 40 ng/ml, increasing to only 3053 ± 1039 ng/ml by 2 hours ($p < 0.05$ vs control).

Thus surface treatment markedly reduced C3a generation at the lower dose of circulating heparin.

Fibrinopeptide A. At full heparinization (5 U/ml), fibrinopeptide A levels (Fig. 7) remained low in both the untreated and coated circuits (88 ± 49 ng/ml control vs 68 ± 48 ng/ml coated at 2 hours). Importantly, fibrinopeptide A levels with 1 U/ml heparin doses were markedly elevated in both the control and treated circuits at 2 hours (793 ± 337 ng/ml control vs 563 ± 459 ng/ml coated; $p > 0.05$).

These data indicate that the surface treatment did not provide effective anticoagulant activity in this in vitro model.

Discussion

Effects on platelets. The artificial surface of cardiopulmonary bypass circuits is a potent stimulus for platelet activation, as has been described in both clinical[24, 25] and experimental studies.[10, 26] Platelet adhesion to the surface and subsequent activation are thought to be mediated by specific receptor-ligand interactions of the platelet membrane with surface-bound fibrinogen.[27] Moreover, the platelet

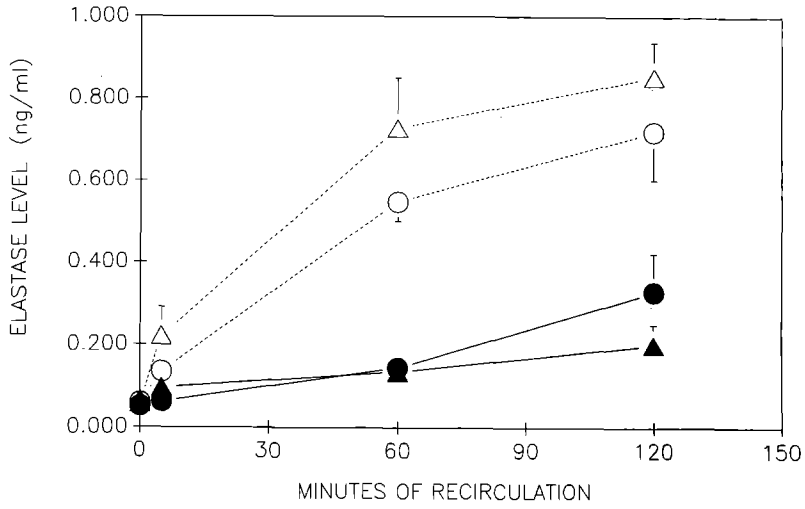

Fig. 5. Neutrophil elastase. Mean levels of plasma elastase–α_1–proteinase inhibitor complex (mean ± standard error of the mean) are plotted against time and expressed in nanograms per milliliter. Otherwise legend is identical to that of Fig. 1.

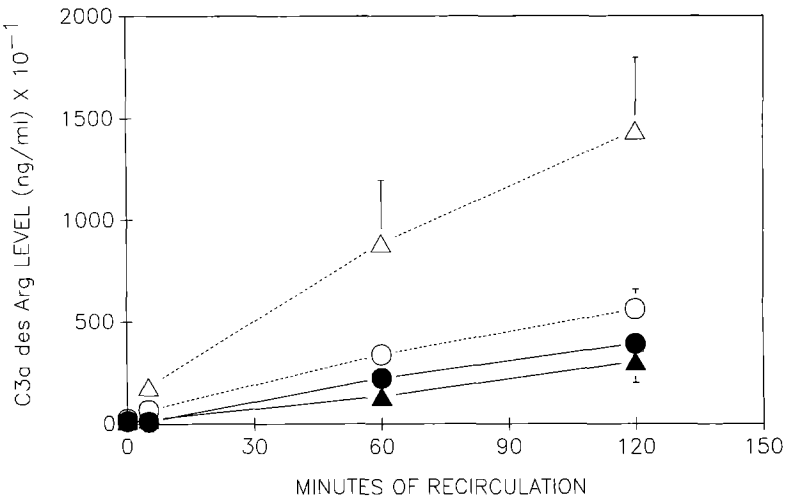

Fig. 6. Complement C3a des Arg. Mean plasma C3a des Arg levels (mean ± standard error of the mean) are plotted against time and expressed in nanograms per milliliter × 10^{-1}. Otherwise legend is identical to that of Fig. 1.

membrane glycoprotein IIb/IIIa complex is the receptor responsible for adhesion.[28] Platelet activation is customarily viewed as an organized process resulting in shape change, granule release, and thromboxane generation. Recent data have suggested, however, that the platelet release reaction observed in the extracorporeal circuit may be the result of shear force–induced destruction of surface adherent platelets.[29]

In these experiments, the heparin-bonded surface attenuated early platelet depletion and markedly reduced platelet granule release, as reflected in the measured levels of platelet factor 4 and β-thromboglobulin. This effect was observed at both levels of heparinization. Although there was a tendency toward reduced generation of thromboxanes in both groups of coated circuits, this was more pronounced with the lower dose of heparin. Interestingly, the surface treatment had a transient effect on platelet adhesion, whereas it caused a sustained reduction in platelet granule release. This uncoupling of adhesion and degranulation was observed by Musial and

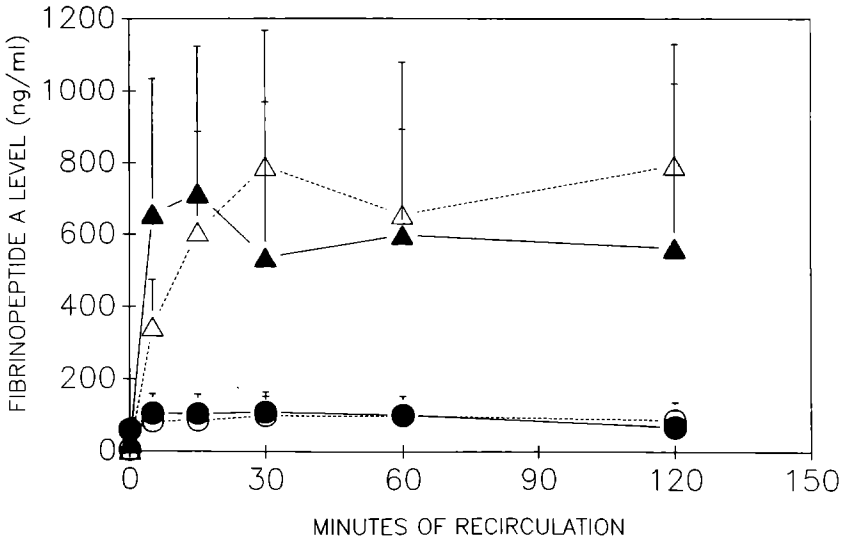

Fig. 7. Fibrinopeptide A. Mean plasma fibrinopeptide A levels (mean ± standard error of the mean) are plotted against time and expressed in nanograms per milliliter. Otherwise legend is identical to that of Fig. 1.

associates[29] when disintegrins were introduced into the extracorporeal circuit. It may be the case that altered surface composition has at least a transient effect on the plasma protein coat.[30] This, in turn, may affect the time course and extent of platelet adhesion and activation in this model. Artificial surface exposure is known to induce stereotypic changes in the platelet receptor population, with demonstrable effects on both the glycoprotein IIb/IIIa complex and the α_2-adrenergic receptor.[31] Thus an altered surface may present a quantitatively, if not qualitatively, different stimulus to the platelet population, thereby fundamentally altering the dynamic platelet-surface interaction.

Other investigators have reported that heparin surface treatment had a sustained impact on platelet adhesion during in vitro surface contact.[32] It is possible that differences in recirculation temperature and blood dilution may account for the differences observed in the circulating platelet count in our experiments compared with those of Videm and associates.[32]

It is also noteworthy that the onset of platelet dysfunction, as assessed by ADP-induced aggregation, was delayed in these studies by surface treatment. This effect was apparent at the higher concentration of heparin. Given that this in vitro model of cardiopulmonary bypass presents a substantial injurious stimulus to platelets, these data suggest a promising role for surface modification in reducing platelet activation.

Effects on the inflammatory response. Activated leukocytes have a central role in the pathophysiology of cardiopulmonary bypass. The most common complication is prolonged ventilator dependence and is known to result from lung microvascular injury effected by the neutrophil.[5, 6, 33] Contact system activation generates both complement[34, 35] and kallikrein.[36, 37] Levels of C3a are known to rise progressively in blood during exposure to oxygenators.[38, 39] Furthermore, C5a, C3b, and kallikrein directly activate neutrophils,[34] thereby establishing the nexus between the cellular and humoral limbs of the systemic inflammatory response. Neutrophil activation in this context is characterized by release of elastase, a serine protease contained in the "azurophilic" granule, and release of lactoferrin, a "specific" granule marker.[33, 40]

This model of simulated cardiopulmonary bypass manifested a mild, but consistent decrease in circulating leukocyte count, a change that was more pronounced at reduced heparin concentrations. The surface treatment reduced leukocyte depletion, with a greater impact observed at the lower heparin concentration. Elastase release was used as a marker of neutrophil activation and was found to be significantly reduced at both concentrations of heparin in the experimental circuits. In addition, although the surface alteration reduced C3a generation, a significant effect was observed only at the lower level of circulating heparin. Taken together, these data suggest that surface treatment reduced

leukocyte activation independent of its effects on C3a production. Indeed, reduction in complement activation in this in vitro model may be solely dependent on heparin concentration, whether in fluid phase or bound to the surface, and is supported by the literature.[41-43] This is in contrast to clinical data reflecting increased complement generation after the start of cardiopulmonary bypass despite requisite use of heparin.[6, 35] The differences are most likely related to in vivo factors that are not present in our closed in vitro model. Other humoral mediators, such as kallikrein, may therefore promote leukocyte activation in this model.[44]

Videm and colleagues[22] have proposed that surface biocompatibility might best be assessed by the use of indices of leukocyte activation. This recognizes that the leukocyte, as mediator of microvascular injury, serves as an end point of the complement and kallikrein systems in the manifestation of the inflammatory response.[44] In addition, platelet activation during blood surface contact may stimulate leukocytes, in that activated platelets have been shown to adhere to both monocytes and neutrophils by a specific receptor.[45]

Although heparin surface treatment was originally developed to reduce the requirement for exogenous heparin administration, the data from these studies and other investigations indicate that the surface has a consistent effect on complement and leukocyte activation.[23, 32, 46, 47] Our results suggest that the antiinflammatory activity of the Carmeda surface treatment is not contingent on a reduction in the exogenous heparin dose.

Effects on blood coagulation. Since the development of the first extracorporeal circuit, the most significant clinical problems after cardiopulmonary bypass have been related to hemorrhage.[2] Heparin coating of the circuit was proposed as a nonthrombogenic interface that would reduce reliance on high-dose exogenous heparin during blood contact with the artificial surface. This, in turn, would be expected to limit perioperative blood loss and subsequent transfusion requirements, as well as prevent unnecessary reoperation.[48-50] Initial attempts at heparin surface bonding were disappointing, with no surface tested heretofore demonstrating any real anticoagulant activity.[8] Although studies by von Segesser and associates[51, 52] have used heparin-coated oxygenators and pumps without exogenous heparin administration, these studies were performed in animals and are not entirely relevant to clinical cardiopulmonary bypass. The Carmeda treatment is the result of new bonding technology, which is thought to provide good active site orientation of the heparin molecule, relative to the fluid component of the blood. It has also been demonstrated to have in vitro thrombin-inhibiting activity.[8]

The data obtained in these experiments, using human blood, indicate that the Carmeda surface treatment did not affect generation of fibrinopeptide A at either dose of heparin. Importantly, this is the only study in which the anticoagulant effects of surface modification are assessed with human blood using a sensitive index of fibrinogen cleavage, in the absence of full heparinization. Although some clinical data indicate that the Duraflo heparin treatment (Baxter Healthcare Corp., Irvine, Calif.) reduced requirements for protamine administration and blood transfusion, with reduction in fibrinolysis as well, the study reporting these data used aprotinin in the low heparin treatment group but not in the group of patients receiving full heparinization.[49] In addition, Jones and colleagues[53] recently published results of a clinical trial using the Carmeda surface for cardiopulmonary bypass in patients at high risk for systemic heparinization. They were able to reduce exogenous administration of heparin and consequently protamine without any overt thrombotic complications, although fibrinopeptide A levels were not measured in this study.

We did not observe any evidence of gross clot in the circuits in which a reduced heparin dose was used, with or without the Carmeda surface treatment. This fact, in concert with the fibrinopeptide A data, would suggest that the absence of adverse clotting noted in clinical studies using heparin surface treatment may not be attributable to the heparin coating per se.[48-50, 54, 55] These clinical studies have, of course, lacked the control group using reduced heparinization in the absence of surface modification. In addition, the question is raised as to whether this surface treatment has effective anticoagulant activity when a large surface area, such as an oxygenator, constitutes the thrombogenic stimulus. Our experiments directly address this question with human blood in a well-established model of cardiopulmonary bypass.

Summary

The data indicate that heparin surface treatment had a significant impact on the extent and time course of several indices of platelet and leukocyte activation. Surface treatment ameliorated formed blood element injury, with effects noted, generally,

at full or reduced heparinization. The data do not indicate that surface treatment reduced fibrinogen cleavage. Thus the Carmeda surface treatment did not exhibit anticoagulant efficacy, yet it did manifest significant antiinflammatory activity in this model. Our results, along with those obtained by other investigators evaluating the most recent generation of heparin surface treatments, continue to validate the concept that surface modification may yet provide a more biocompatible surface than has thus far been realized. Perhaps surface application of inhibitors of contact system initiation, such as factor XII, as suggested by Edmunds, Niewiarowski, and Colman,[44] may yet provide more meaningful control of the surface-induced inflammatory response.

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